Retinal ganglion cell loss evaluation by flow cytometry after intravitreal injection of endothelin-1 in the rat

Bertrand Bardet, Anabelle Sequeira-Le Grand, Niyazi Acar, Catherine P. Garcher, Alain M. Bron, Lionel Brétillon

6127—A509

Retinal Ganglion Cell Loss Evaluation by Flow Cytometry After Intravitreal Injection of Endothelin-1 in the Rat

B. Bardet[1], A. Sequeira-Le Grand[2], N. Acar[1], C. P. Garcher[3,1], A. M. Bron[3,1] and L. Bretillon[1]

[1]INRA, University of Burgundy, Eye & Nutrition Research Group, Dijon, France
[2]Flow Cytometry Department, University of Burgundy, Dijon Cedex, France
[3]Ophthalmology, University Hospital, Dijon, France

Commercial Relationships: B. Bardet, None; **A. Sequeira-Le Grand**, None; **N. Acar**, None; **C.P. Garcher**, None; **A.M. Bron**, None; **L. Bretillon**, None.

Support: None.

Abstract

Purpose: Retinal Ganglion Cell (RGC) quantification is a critical point to assess the relevance of an animal model of glaucoma or the efficacy of drugs that target RGC degeneration. The aim of the present study was to evaluate the feasibility and the reliability of flow cytometry as a new tool for counting RGCs.

Methods:RGC loss was induced in Wistar rats (n=10) by intravitreal injection of 5µL of 500µM endothelin-1. The controlateral eye was used as a control. A control group (n=9) received a 5µL-intravitreal injection of saline. After one week, retinas were harvested and manually homogenized in order to obtain a single-cell suspension. Primary anti-Thy1.1 (CD90) antibody was used as a marker for RGCs. A secondary fluorescent antibody was subsequently incubated. Negative control incubations were performed with non relevant IgG as primary antibody. Thereafter, cells were rinsed and resuspended in PBS before analysis by flow cytometry. Another group of rats (n=10) was investigated by Fluorogold retrolabelling after intravitreal injection of endothelin-1. The Fluorogold staining was evaluated by flow cytometry.

Results:RGCs loss after 5µL intravitreal injection of 500µM endothelin-1 was 24.2 ± 6.7% compared with controlateral eye. No RGC loss was observed in the sham operated group. The coefficient of variation for this technique was from 1.1% to 3.1% in controls. The specificity of this method for counting RGC was confirmed by assessing that Thy1.1 positive cells were also labelled with Fluorogold.

Conclusions: Based on our preliminary findings, we suggest that flow cytometry is a convenient, rapid and reliable technique for relative quantification of RGC loss in rat models of glaucoma.

Keywords: ganglion cells • flow cytometry